

# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference <b>N.76101A JCI</b>	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. <b>PCT/GB00/00559</b>	International filing date ( <i>day/month/year</i> ) <b>17/02/2000</b>	Priority date ( <i>day/month/year</i> ) <b>17/02/1999</b>
International Patent Classification (IPC) or national classification and IPC <b>A61K39/39</b>		
Applicant <b>ISIS INNOVATION LIMITED et al.</b>		



1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
  
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
 

☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand  <b>15/09/2000</b>	Date of completion of this report  <b>18.05.2001</b>
Name and mailing address of the international preliminary examining authority:  <b>European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465</b>	Authorized officer  <b>Hoesel, H</b>  Telephone No. +49 89 2399 8693 

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# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>N.76101A JCI</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/GB 00/ 00559</b>	International filing date (day/month/year) <b>17/02/2000</b>	(Earliest) Priority Date (day/month/year) <b>17/02/1999</b>
Applicant  <b>ISIS INNOVATION LIMITED et al.</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

### 1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing :



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of Invention is lacking (see Box II).

4. With regard to the title,



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

5. With regard to the abstract,



the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.



None of the figures.

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## INTERNATIONAL SEARCH REPORT

International Application No

P B 00/00559

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K39/39 C12N5/06 C12N5/08 A61K39/00 A61K35/14  
 C07K14/705 C12N15/12 C07K16/28 G01N33/50 A61P37/04  
 A61P37/06 //C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, MEDLINE, CHEM ABS Data, EMBASE, SCISEARCH

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DEAGLIO S: "Human CD38 ligand: A 120-KDa protein predominantly expressed on endothelial cells" JOURNAL OF IMMUNOLOGY, vol. 156, 1996, pages 727-734, XP002146231 cited in the application the whole document	1-18
A	ELLIS JONATHAN H ET AL: "Engineered anti-CD38 monoclonal antibodies for immunotherapy of multiple myeloma." JOURNAL OF IMMUNOLOGY, vol. 155, no. 2, 1995, pages 925-937, XP002146232 ISSN: 0022-1767 the whole document	1,2



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

Date of the actual completion of the international search

11 September 2000

Date of mailing of the international search report

26/09/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+31-70) 340-3016

Authorized officer

Stein, A

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## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 00/00559

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GROUARD G. ET AL: "Regulation of human B cell activation by follicular dendritic cell and T cell signals." CURRENT TOPICS IN MICROBIOLOGY AND IMMUNOLOGY, (1995) 201/- (105-117). , XP000938968 the whole document ----	7-11
A	WYKES M ET AL: "Dendritic cells interact directly with naive B lymphocytes to transfer antigen and initiate class switching in a primary T-dependent response" JOURNAL OF IMMUNOLOGY, vol. 161, 1998, pages 1313-1319, XP002146233 cited in the application the whole document ----	7-11
A	GB 2 294 321 A (YAMANOUCHI RESEARCH INST ;IMP CANCER RESEARCH FUND (GB)) 24 April 1996 (1996-04-24) the whole document ----	12-18
A	HORENSTEIN A ET AL: "CD38 binding to human myeloid cells is mediated by mouse and human CD31" BIOCHEM J, vol. 330, 1998, pages 1129-1135, XP002146234 cited in the application the whole document -----	12-18

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/00559

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2294321 A	24-04-1996	NONE	

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## PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 22 MAY 2001

WIPO

PCT

161

Applicant's or agent's file reference N.76101A JCI	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB00/00559	International filing date (day/month/year) 17/02/2000	Priority date (day/month/year) 17/02/1999
International Patent Classification (IPC) or national classification and IPC A61K39/39		
Applicant ISIS INNOVATION LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



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These annexes consist of a total of sheets.

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- I ☒ Basis of the report
- II ☐ Priority
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- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand  15/09/2000	Date of completion of this report  18.05.2001
Name and mailing address of the international preliminary examining authority:   European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer  Hoesel, H  Telephone No. +49 89 2399 8693  

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# PATENT COOPERATION TREATY

## PCT

### NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
United States Patent and Trademark  
Office  
Box PCT  
Washington, D.C.20231  
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

<b>Date of mailing (day/month/year)</b> 25 October 2000 (25.10.00)	
<b>International application No.</b> PCT/GB00/00559	<b>Applicant's or agent's file reference</b> N.76101A JCI
<b>International filing date (day/month/year)</b> 17 February 2000 (17.02.00)	<b>Priority date (day/month/year)</b> 17 February 1999 (17.02.99)
<b>Applicant</b> MACPHERSON, George, Gordon et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:  
15 September 2000 (15.09.00)

☐ in a notice effecting later election filed with the International Bureau on:  
 \_\_\_\_\_

2. The election ☒ was

☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	<b>Authorized officer</b> <p style="text-align: center;">Olivia TEFY</p>
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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J. A. KEMP

## PATENT COOPERATION TREATY

22 MAY 2000

JCI/SA PCT

NOTIFICATION CONCERNING  
SUBMISSION OR TRANSMITTAL  
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

From the INTERNATIONAL BUREAU

To:

IRVINE, Jonquil, Claire  
J.A. Kemp & Co.  
14 South Square  
Gray's Inn  
London WC1R 5LX  
ROYAUME-UNI

Date of mailing (day/month/year) 10 May 2000 (10.05.00)	
Applicant's or agent's file reference N.76101A JCI	IMPORTANT NOTIFICATION
International application No. PCT/GB00/00559	International filing date (day/month/year) 17 February 2000 (17.02.00)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 17 February 1999 (17.02.99)
Applicant ISIS INNOVATION LIMITED et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
3. An asterisk(\*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
4. The letters "NR" appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

<u>Priority date</u>	<u>Priority application No.</u>	<u>Country or regional Office or PCT receiving Office</u>	<u>Date of receipt of priority document</u>
17 Febr 1999 (17.02.99)	9903664.2	GB	01 May 2000 (01.05.00)

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Tessadel PAMPLIEGA *Tdp*

Telephone No. (41-22) 338.83.38

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# PCT

## REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference  
(if desired) (12 characters maximum) N.76101A JCI

Box No. I TITLE OF INVENTION	
ADJUVANT AND CELL MATURATION AGENT	
Box No. II APPLICANT	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) ISIS INNOVATION LIMITED Ewert House Ewert Place Summertown Oxford OX2 7BZ	
<input type="checkbox"/> This person is also inventor.	
Telephone No.	
Facsimile No.	
Teleprinter No.	
State (that is, country) of nationality: GB	State (that is, country) of residence: GB
This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input checked="" type="checkbox"/> all designated States except the United States of America <input type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box	
Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) MACPHERSON George Gordon University of Oxford Sir William Dunn School of Pathology South Parks Road Oxford OX1 3RE United Kingdom	
This person is: <input type="checkbox"/> applicant only <input checked="" type="checkbox"/> applicant and inventor <input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)	
State (that is, country) of nationality: GB	State (that is, country) of residence: GB
This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input checked="" type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box	
<input type="checkbox"/> Further applicants and/or (further) inventors are indicated on a continuation sheet.	
Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE	
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: <input checked="" type="checkbox"/> agent <input type="checkbox"/> common representative	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) IRVINE, Jonquil Claire J.A. KEMP & CO., 14 South Square, Gray's Inn, London, WC1R 5LX, United Kingdom.	
Telephone No.	
+44 171 405 3292	
Facsimile No.	
+44 171 242 8932	
Teleprinter No.	
23676	
<input type="checkbox"/> Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.	

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## Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

WYKES Michelle Niloufer  
Mater Medical Research Institute  
Level 3 Aubigny Place  
Raymond Terrace  
South Brisbane  
Queensland 4152  
Australia

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:  
AUState (that is, country) of residence:  
AU

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

- ☐ applicant only  
☐ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

- ☐ applicant only  
☐ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

- ☐ applicant only  
☐ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

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## Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked).

## Regional Patent

- ☒ AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

## National Patent (if other kind of protection or treatment desired, specify on dotted line):

- |  |  |
|--|--|
| <input checked="" type="checkbox"/> AE United Arab Emirates                  | <input checked="" type="checkbox"/> LR Liberia                                   |
| <input checked="" type="checkbox"/> AL Albania                               | <input checked="" type="checkbox"/> LS Lesotho                                   |
| <input checked="" type="checkbox"/> AM Armenia                               | <input checked="" type="checkbox"/> LT Lithuania                                 |
| <input checked="" type="checkbox"/> AT Austria                               | <input checked="" type="checkbox"/> LU Luxembourg                                |
| <input checked="" type="checkbox"/> AU Australia                             | <input checked="" type="checkbox"/> LV Latvia                                    |
| <input checked="" type="checkbox"/> AZ Azerbaijan                            | <input checked="" type="checkbox"/> MD Republic of Moldova                       |
| <input checked="" type="checkbox"/> BA Bosnia and Herzegovina                | <input checked="" type="checkbox"/> MG Madagascar                                |
| <input checked="" type="checkbox"/> BB Barbados                              | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia |
| <input checked="" type="checkbox"/> BG Bulgaria                              | <input checked="" type="checkbox"/> MA Morocco                                   |
| <input checked="" type="checkbox"/> BR Brazil                                | <input checked="" type="checkbox"/> MN Mongolia                                  |
| <input checked="" type="checkbox"/> BY Belarus                               | <input checked="" type="checkbox"/> MW Malawi                                    |
| <input checked="" type="checkbox"/> CA Canada                                | <input checked="" type="checkbox"/> MX Mexico                                    |
| <input checked="" type="checkbox"/> CH and LI Switzerland and Liechtenstein  | <input checked="" type="checkbox"/> NO Norway                                    |
| <input checked="" type="checkbox"/> CN China                                 | <input checked="" type="checkbox"/> NZ New Zealand                               |
| <input checked="" type="checkbox"/> CU Cuba                                  | <input checked="" type="checkbox"/> PL Poland                                    |
| <input checked="" type="checkbox"/> CZ Czech Republic                        | <input checked="" type="checkbox"/> PT Portugal                                  |
| <input checked="" type="checkbox"/> DE Germany                               | <input checked="" type="checkbox"/> RO Romania                                   |
| <input checked="" type="checkbox"/> DK Denmark                               | <input checked="" type="checkbox"/> RU Russian Federation                        |
| <input checked="" type="checkbox"/> EE Estonia                               | <input checked="" type="checkbox"/> SD Sudan                                     |
| <input checked="" type="checkbox"/> ES Spain                                 | <input checked="" type="checkbox"/> SE Sweden                                    |
| <input checked="" type="checkbox"/> FI Finland                               | <input checked="" type="checkbox"/> SG Singapore                                 |
| <input checked="" type="checkbox"/> GB United Kingdom                        | <input checked="" type="checkbox"/> SI Slovenia                                  |
| <input checked="" type="checkbox"/> GD Grenada                               | <input checked="" type="checkbox"/> SK Slovakia                                  |
| <input checked="" type="checkbox"/> GE Georgia                               | <input checked="" type="checkbox"/> SL Sierra Leone                              |
| <input checked="" type="checkbox"/> GH Ghana                                 | <input checked="" type="checkbox"/> TJ Tajikistan                                |
| <input checked="" type="checkbox"/> GM Gambia                                | <input checked="" type="checkbox"/> TM Turkmenistan                              |
| <input checked="" type="checkbox"/> HR Croatia                               | <input checked="" type="checkbox"/> TR Turkey                                    |
| <input checked="" type="checkbox"/> HU Hungary                               | <input checked="" type="checkbox"/> TT Trinidad and Tobago                       |
| <input checked="" type="checkbox"/> ID Indonesia                             | <input checked="" type="checkbox"/> UA Ukraine                                   |
| <input checked="" type="checkbox"/> IL Israel                                | <input checked="" type="checkbox"/> UG Uganda                                    |
| <input checked="" type="checkbox"/> IN India                                 | <input checked="" type="checkbox"/> US United States of America                  |
| <input checked="" type="checkbox"/> IS Iceland                               | <input checked="" type="checkbox"/> TZ Tanzania                                  |
| <input checked="" type="checkbox"/> JP Japan                                 | <input checked="" type="checkbox"/> UZ Uzbekistan                                |
| <input checked="" type="checkbox"/> KE Kenya                                 | <input checked="" type="checkbox"/> VN Viet Nam                                  |
| <input checked="" type="checkbox"/> KG Kyrgyzstan                            | <input checked="" type="checkbox"/> YU Yugoslavia                                |
| <input checked="" type="checkbox"/> KP Democratic People's Republic of Korea | <input checked="" type="checkbox"/> ZA South Africa                              |
| <input checked="" type="checkbox"/> KR Republic of Korea                     | <input checked="" type="checkbox"/> ZW Zimbabwe                                  |
| <input checked="" type="checkbox"/> KZ Kazakhstan                            |  |
| <input checked="" type="checkbox"/> LC Saint Lucia                           |  |
| <input checked="" type="checkbox"/> LK Sri Lanka                             |  |

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

- ☒ CR Costa Rica
- ☒ DM Dominica

**Precautionary Designation Statement:** In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

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**Supplemental Box** *If the Supplemental Box is not used, this sheet should not be included in the request.*

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

- (i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;
- (ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;
- (iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;
- (iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;
- (v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;
- (vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;
- (vii) if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

#### Continuation of Box No. IV

GOLDIN, Douglas Michael; ELLIS-JONES, Patrick George Armine; BARLOW, Roy James; SENIOR, Alan Murray; BENTHAM, Stephen; AYERS, Martyn Lewis Stanley; WOODS, Geoffrey Corlett; CRESSWELL, Thomas Anthony; SEXTON, Jane Helen; NICHOLLS, Michael John; MARSHALL Monica Anne; WEBB, Andrew John; KEEN, Celia Mary; PRICE, Nigel John King; IRVINE, Jonquil Claire; LEEMING, John Gerard; DUCKWORTH, Timothy John; MCCLUSKIE, Gail Wilson; WRIGHT, Simon Mark; CURWEN, Julian Charles Barton; CLEEVE, James Harold Findlay; SMITH, Samuel Leonard; BENSON, John Everett, CAMPBELL Patrick John; MERRYWEATHER, Colin Henry; DUCKETT, Anthony Joseph; BENTHAM, Andrew; and ROQUES, Sarah Elizabeth; SRINIVASAN, Ravi Chandran; TYSON, Robin Edward and BURNSIDE Ivan John of: J.A. KEMP & CO., 14 South Square, Gray's Inn, London, WC1R 5LX, United Kingdom.

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<b>Box No. VI PRIORITY CLAIM</b>					<input type="checkbox"/> Further priority claims are indicated in the Supplemental Box.
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:			
		national application: country	regional application: regional Office	international application: receiving Office	
item (1) 17 February 1999	9903664.2	UK			
item (2)					
item (3)					
<input type="checkbox"/> The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s):					
<small>* Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.</small>					
<b>Box No. VII INTERNATIONAL SEARCHING AUTHORITY</b>					
Choice of International Searching Authority (ISA) <small>(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):</small>		Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority): Date (day/month/year)      Number      Country (or regional Office)			
ISA /					
<b>Box No. VIII CHECK LIST; LANGUAGE OF FILING</b>					
This international application contains the following number of sheets: request : 5 description (excluding sequence listing part) : 25 claims : 3 abstract : 1 drawings : 10 sequence listing part of description : Total number of sheets : 44		This international application is accompanied by the item(s) marked below: 1. <input type="checkbox"/> fee calculation sheet 2. <input type="checkbox"/> separate signed power of attorney 3. <input type="checkbox"/> copy of general power of attorney; reference number, if any: 4. <input type="checkbox"/> statement explaining lack of signature 5. <input type="checkbox"/> priority document(s) identified in Box No. VI as item(s): 6. <input type="checkbox"/> translation of international application into (language): 7. <input type="checkbox"/> separate indications concerning deposited microorganism or other biological material 8. <input type="checkbox"/> nucleotide and/or amino acid sequence listing in computer readable form 9. <input type="checkbox"/> other (specify):			
Figure of the drawings which should accompany the abstract:		Language of filing of the international application: English			
<b>Box No. IX SIGNATURE OF APPLICANT OR AGENT</b>					
Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).          _____ ELLIS-JONES, Patrick George Armine					

For receiving Office use only	
1. Date of actual receipt of the purported international application:  3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:  4. Date of timely receipt of the required corrections under PCT Article 11(2):  5. International Searching Authority (if two or more are competent): ISA /	2. Drawings:  <input type="checkbox"/> received:  <input type="checkbox"/> not received:  6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid.

For International Bureau use only
Date of receipt of the record copy by the International Bureau:

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# PATENT COOPERATION TREATY

PCT

## NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

From the INTERNATIONAL BUREAU

To:

IRVINE, Jonquil, Claire  
J.A. Kemp & Co.  
14 South Square  
Gray's Inn  
London WC1R 5LX  
ROYAUME-UNI

Date of mailing (day/month/year) 24 August 2000 (24.08.00)		IMPORTANT NOTICE	
Applicant's or agent's file reference N.76101A JCI			
International application No. PCT/GB00/00559	International filing date (day/month/year) 17 February 2000 (17.02.00)	Priority date (day/month/year) 17 February 1999 (17.02.99)	
Applicant ISIS INNOVATION LIMITED et al			

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:  
AU, KP, KR, US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

AE, AL, AM, AP, AT, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EA, EE, EP, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, OA, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on 24 August 2000 (24.08.00) under No. WO 00/48631

### REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

### REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

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# PATENT COOPERATION TREATY

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

IRVINE, Jonquil C.  
J.A. KEMP & CO.  
14 South Square  
Gray's Inn  
London WC1R 5LX  
GRANDE BRETAGNE

**J. A. KEMP & Co**

**REC'D 21 MAY 2001**

Action by.....

**PCT**

NOTIFICATION OF TRANSMITTAL OF  
THE INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing  
(day/month/year)

18.05.2001

Applicant's or agent's file reference  
N.76101A JCI

## IMPORTANT NOTIFICATION

International application No.  
PCT/GB00/00559

International filing date (day/month/year)  
17/02/2000

Priority date (day/month/year)  
17/02/1999

Applicant

ISIS INNOVATION LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

#### 4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/

 European Patent Office  
D-80298 Munich  
Tel. +49 89 2399 - 0 Tx: 523656 epmu d  
Fax: +49 89 2399 - 4465

Authorized officer

Neumann, M

Tel. +49 89 2399-7351



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**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/GB00/00559

**I. Basis of the report**

1. With regard to the elements of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):  
Description, pages:

1-25 as originally filed

**Claims, No.:**

1-18 as originally filed

**Drawings, sheets:**

1/12-12/12 as originally filed

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).  
☐ the language of publication of the international application (under Rule 48.3(b)).  
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.  
☐ filed together with the international application in computer readable form.  
☐ furnished subsequently to this Authority in written form.  
☐ furnished subsequently to this Authority in computer readable form.  
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:

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**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/GB00/00559

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 12 (partly), 13, 14, 16 - 18.

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 12 (partly), 13, 14, 16 - 18 are so unclear that no meaningful opinion could be formed (*specify*):  
see separate sheet
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☐ no international search report has been established for the said claims Nos. .
2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:
- ☐ the written form has not been furnished or does not comply with the standard.
- ☐ the computer readable form has not been furnished or does not comply with the standard.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)

Yes: Claims 1 - 11, 12, 15

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**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/GB00/00559

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	No:	Claims	
Inventive step (IS)	Yes:	Claims	1 - 11, 12, 15
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1 - 11, 12, 15
	No:	Claims	

2. Citations and explanations  
**see separate sheet**

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:  
**see separate sheet**

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Reference is made to the following documents:

D1: DE 198 02 540 C1

D2: WO 9853048 A

D3: WO 9110683 A

### SECTION III:

1. Claim 1 attempts to define a ligand in negative terms, i.e. in terms of what it is assumed and hoped not to be ("The native ligand, ..., excluding CD31"), as a main feature. This definition does not enable a skilled person to unambiguously identify a said antigen. The claim in addition embraces **homologues** (at least 70%) and **functionally equivalent fragments** of a said ligand (definitions (ii) and (iii)).

According to the description, the characterization of said ligand is limited to a western blot of membrane extracts of lysed dendritic cells ("DC") with a fusion construct comprising CD38 (p. 22, lines 15 - 21). The example is silent as to which of the bands identified represents the "native" DC ligand for CD38. Although it can be concluded with respect to the different expression of the present CD38 ligand and CD31 on DC, B- and T-cells, that the two proteins are not identical, it cannot be excluded that they represent homologues, or at least exhibit a homologous CD38 binding domain.

In the absence of clearly identifiable positive terms and of a sequence analysis, it cannot be assessed whether CD31, **isoforms or fragments thereof** such as known from D5 (abstract and claims 1 - 12) fall within the terms of definitions (ii) and (iii) of present claim 12.

An assessment whether the claimed subject-matter is novel and inventive is therefore possible only with regard to the CD38 ligand as such (i.e. option (i) of claim 12)

2. The objection analogously applies to claims 13 and 14 which relate to a polynucleotide encoding the desired ligand or antibody specific for either the said ligand, homologue or fragment thereof. In the absence of an identification and

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characterization of the ligand, it cannot be assessed whether the polynucleotides covered by claims 15 - 22 of D3 fall within the scope of claim 13 and whether claim 14 embraces known antibodies reacting with fragments or isoforms of CD31.

3. Claims 16 - 18 concern the medical use of an inhibitor that is defined only in terms of a desired function as to inhibit the interaction between CD38 and its receptor on dendritic cells ("DC"). It is not possible to correlate a particular biochemical structure with a said inhibitor, the more as neither the CD38 binding ligand on DC nor the mechanism of interaction and signal transduction have been elucidated. It cannot therefore be excluded that known immunosuppressing agents exert their pharmacological activity via inhibition of this maturation pathway.

Thus, in the absence of any structural definition of a said inhibitor in the application documents, the scope of the said claims especially of claim 17 cannot be assessed with respect to novelty and inventive step.

#### **SECTION V:**

4. The prior art taken into consideration is silent as to the impact of CD38 on maturation of FDC. Consequently, the second medical use according to claims 1 and 2 and the ex vivo maturation method according to claims 3 and 4 are novel and inventive as required by Art. 33(2) and (3) PCT.
5. The prior art does not teach or suggest a vaccine that comprises in combination an antigen and CD38 or functional derivative thereof.

Thus, the subject-matter of claims 5 and 6 also seems to meet the requirements of Art. 33(2) and (3) PCT.

6. The adjuvant activity of immature and mature DC is known. Preparations of mature dendritic cells have been disclosed for immunotherapy in conjunction with ex vivo and in vivo activation of T-cells (D1, p. 2, lines 22 - 31 and 52 - 68; D2, p. 34, line 11 - p. 37, line 26, claims 45 - 49).

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**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

---

International application No. PCT/GB00/00559

Contrary to known methods of promoting DC maturation (cf. D1, p. 4, lines 20 - 28 and line 63 - p. 5, line 15; D2, p. 13, line 10 - p. 14 line 5), the use CD38 as maturation promoter results in DC with significantly reduced B7.1 expression. In spite of this, the cells are effective in activating cytotoxic T-cells.

Thus, the DC preparations of claim 7 and their medical uses as covered by claims 8 - 11, are considered to be novel and inventive in view of the relevant prior art.

7. The prior art is silent as to an additional ligand for CD38 which is particularly expressed on follicular dendritic cells. Thus, the ligand as defined in claim 12, option (1) is novel and inventive in the sense of art. 33(2) and (3) PCT.
8. The method of claim 15 satisfies the requirements of Art. 33(2) and (3) PCT, since the prior art neither discloses nor suggests a screening method that is based on monitoring a competition of an unknown agent with the dendritic cell ligand for CD38 for binding to CD38.

Claim 15 thus appears to meet the requirements of Art. 33(2) and (3) PCT.

**SECTION VII:**

9. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 - D3 is not mentioned in the description, nor are these documents identified therein.

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# PATENT COOPERATION TREATY

(SA)

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

IRVINE, Jonquil C.  
J.A. KEMP & CO.  
14 South Square  
Gray's Inn  
London WC1R 5LX  
GRANDE BRETAGNE

**J. A. KEMP & Co**

- 4 OCT 2000

Action by \_\_\_\_\_

**NOTIFICATION OF RECEIPT  
OF DEMAND BY COMPETENT INTERNATIONAL  
PRELIMINARY EXAMINING AUTHORITY**

(PCT Rules 59.3(e) and 61.1(b), first sentence  
and Administrative Instructions, Section 601(a))

Date of mailing  
(day/month/year)

04.10.00

Applicant's or agent's file reference

N.76101A JCI

**IMPORTANT NOTIFICATION**

International application No.

PCT/GB 00/ 00559

International filing date (day/month/year)

17/02/2000

Priority date (day/month/year)

17/02/1999

Applicant

ISIS INNOVATION LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

15/09/2000

2. This date of receipt is:

- ☒ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
- ☐ the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
- ☐ the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. ☐ **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II:

- ☐ (If applicable) This notification confirms the information given by telephone, facsimile transmission or in person on: \_\_\_\_\_

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/

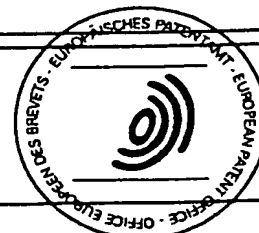


European Patent Office  
D-80298 Munich  
Tel. (+ 49-89) 2399-0, Tx: 523656 epmu d  
Fax: (+ 49-89) 2399-4465

Authorized officer

PITARD J A S

Tel. (+ 49-89) 2399-2156



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The demand must be filed directly with the competent International Preliminary Examining Authority, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ EPO

# PCT

## CHAPTER II

### DEMAND

under Article 31 of the Patent Cooperation Treaty:  
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA		Date of receipt of DEMAND
<b>Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION</b>		Applicant's or agent's file reference N.76101A JCI
International application No. PCT/GB00/00559	International filing date (day/month/year) 17 FEBRUARY 2000	(Earliest) Priority date (day/month/year) 17 FEBRUARY 1999
Title of invention ADJUVANT AND CELL MATURATION AGENT		
<b>Box No. II APPLICANT(S)</b>		
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  ISIS INNOVATION LIMITED Ewert House Ewert Place Summertown Oxford OX2 7BZ United Kingdom		Telephone No.:
		Facsimile No.:
		Teleprinter No.:
State (that is, country) of nationality: GB	State (that is, country) of residence: GB	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  MACPHERSON, George Gordon University of Oxford Sir William Dunn School of Pathology South Parks Road Oxford OX1 3RE United Kingdom		
State (that is, country) of nationality: GB	State (that is, country) of residence: GB	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  WYKES, Michelle Niloufer Mater Medical Research Institute Level 3 Aubigny Place Raymond Terrace South Brisbane Queensland 4152 Australia		
State (that is, country) of nationality: AU	State (that is, country) of residence: AU	
<input type="checkbox"/> Further applicants are indicated on a continuation sheet.		

*This Page Blank (uspto)*

**Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE**

The following person is ☒ agent ☐ common representative  
 and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.  
☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.  
☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

IRVINE, Jonquil Claire  
 J.A. KEMP & CO.,  
 14 South Square,  
 Gray's Inn,  
 London, WC1R 5LX,  
 United Kingdom.

Telephone No.:

+44 20 7405 3292

Facsimile No.:

+44 20 7242 8932

Teleprinter No.:

23676

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

**Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION****Statement concerning amendments:\***

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filed

the description ☐ as originally filed  
☐ as amended under Article 34

the claims ☐ as originally filed  
☐ as amended under Article 19 (together with any accompanying statement)  
☐ as amended under Article 34

the drawings ☐ as originally filed  
☐ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

\* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH

☒ which is the language in which the international application was filed.

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**Box No. V ELECTION OF STATES**

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**Box No. VI CHECK LIST**

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

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|--|---|----------|
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| 2. amendments under Article 34   | : | sheets   |
| 3. copy (or, where required, translation) of amendments under Article 19 | : | sheets   |
| 4. copy (or, where required, translation) of statement under Article 19  | : | sheets   |
| 5. letter  | : | 1 sheets |
| 6. other ( <i>specify</i> )  | : | sheets   |

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The demand is also accompanied by the item(s) marked below:

- |  |   |
|--|---|
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| 2. <input type="checkbox"/> separate signed power of attorney                            | 5. <input type="checkbox"/> nucleotide and or amino acid sequence listing in computer readable form |
| 3. <input type="checkbox"/> copy of general power of attorney; reference number, if any: | 6. <input type="checkbox"/> other ( <i>specify</i> ):   |

**Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE**

*Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).*

ELLIS-JONES, Patrick George Armine

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1. Date of actual receipt of DEMAND: . . . . .
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b): . . . . .
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
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<p>(21) International Application Number: PCT/GB00/00559 (22) International Filing Date: 17 February 2000 (17.02.00) (30) Priority Data: 9903664.2 17 February 1999 (17.02.99) GB (71) Applicant (<i>for all designated States except US</i>): ISIS INNOVATION LIMITED [GB/GB]; Ewert House, Ewert Place, Summertown, Oxford OX2 7BZ (GB). (72) Inventors; and (75) Inventors/Applicants (<i>for US only</i>): MACPHERSON, George, Gordon [GB/GB]; University of Oxford, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE (GB). WYKES, Michelle, Niloufer [AU/AU]; Mater Medical Research Institute, Level 3 Aubigny Place, Raymond Terrace, South Brisbane, QLD 4152 (AU). (74) Agents: IRVINE, Jonquil, Claire et al.; J.A. Kemp &amp; Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).</p>		<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>Without international search report and to be republished upon receipt of that report.</i></p>
<p>(54) Title: ADJUVANT AND CELL MATURATION AGENT</p> <p>(57) Abstract</p> <p>Use of CD38, or a portion thereof or analogue thereof which can inhibit the binding of CD38 to a non-follicular dendritic cell (DC) or a follicular dendritic cell (FDC) and which retains the ability of CD38 to stimulate a DC or FDC, for use in the manufacture of an adjuvant for use in immunotherapy. Also provided is a method of maturing DCs <i>in vitro</i> and a vaccine comprising mature DCs. The invention also provides a natural ligand of CD38 on FDCs and DCs and inhibitors of the ligand. The inhibitors can be used for immunosuppression.</p>		

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ADJUVANT AND CELL MATURATION AGENT

The invention relates to the use of CD38, or a portion or analogue thereof, as an adjuvant or to cause maturation of dendritic cells *in vitro*; and to uses of the mature dendritic cells. The invention also relates to the natural ligand of CD38 on follicular and non-follicular dendritic cells and to a method of identifying adjuvants.

Follicular dendritic cells (FDCs) and non-follicular dendritic cells (DCs) present antigen to T cells and B cells. FDC networks are present in lymph nodes and play a critical role in the development of memory B cells in germinal centres. DCs are important in the development of primary T cell responses, particularly CD8 T cell responses.

Although the role of CD38 has previously been a matter of much investigation, the effect of CD38 on dendritic cells has not been investigated. The inventors have shown that administration of a CD38 construct increases the size and number of FDC networks in germinal centres. This would cause an increased efficiency in antigen presentation to B cells and result in an improvement in long term B cell memory and recall responses.

The inventors have also shown that the CD38 construct causes maturation of DCs. Since immature DCs are better than mature DCs in taking up antigen and mature DCs are better at stimulating primary T cell responses, this property of CD38 can be used when preparing DCs *in vitro*. Immature DCs can be used to take up antigen and then be treated with CD38 to change the phenotype to the mature form. Such mature DCs will be more efficient at stimulating primary T cell responses (for example *in vitro* or after administration to a patient).

Using the CD38 construct the inventors have also identified the natural ligand of CD38 on FDC and DC.

The invention thus provides use of CD38, or a portion thereof or analogue thereof which can inhibit the binding of CD38 to a non-follicular dendritic cell (DC) or a follicular dendritic cell (FDC) and which retains the ability of CD38 to stimulate a DC or FDC, for use in the manufacture of an adjuvant for use in immunotherapy. Such an adjuvant may be administered together with, or separately from, an antigen for immunisation. It may, for example, preferably be administered several days after

immunisation, e.g. 4-5 days, after immunisation.

The invention also provides a method of causing maturation of DCs comprising contacting DCs *ex vivo* with CD38, or a CD38 portion or analogue of the invention. The invention additionally provides an *ex vivo* mature DC that has been

5 made using the method.

The invention provides (i) the native ligand present on a FDC or DC which binds CD38 and which is substantially free of FDC or DC cell membrane, excluding CD31; (ii) a protein which is at least 70% homologous to (i) and binds CD38; or (iii) a fragment of (i) or (ii) which retains the ability to bind CD38. The invention also

10 provides an inhibitor of the same CD38 ligand which specifically inhibits activation of the ligand by CD38.

The invention is illustrated by the accompanying drawings in which:

Figure 1a shows the molecular weight of the CD38/IgG1 construct in reducing (on the right) and non-reducing (on the left) conditions.

15 Figure 1b shows the molecular weight of the construct after N-endoglucosidase treatment.

Figure 2 and Figure 3 show cytometry of DCs.

Figure 4 shows CD38 ligand.

Figure 4a shows MHC class I levels on DCs.

20 Figure 4b shows MHC class II levels on DCs.

Figure 4c shows B7.1 levels on DCs.

Figure 4d shows B7.2 levels on DCs.

Figure 5 shows anti-DNP antibody responses.

Figure 6 shows CTL assay results.

25 The term 'analogue' as used below also includes portions of CD38.

The CD38 used in the invention is generally a mammalian or avian CD38, such as a human, primate or rodent CD38 (e.g. mouse or rat CD38). Thus the CD38 can be any of the different species forms, or any of the naturally occurring allelic forms (including variants in an individual such as splice variants) which occur

30 naturally in such animals. Generally such forms of CD38 will be able to bind FDCs and/or DCs and will be able to stimulate FDCs and/or DCs.



The CD38 may be one which is soluble *in vivo*, e.g. a naturally occurring soluble form. Such naturally-occurring soluble forms have previously been described (see, for example Funaro *et al.*, Int. Immunol. (1996) 8, 1643-1650 and Horenstein *et al.*, Biochem. J. (1998) 330, 1129-1135.)

5           When CD38 (or an analogue with CD38 sequence) is administered to humans according to the invention, it is preferably human CD38 (or preferably the analogue has human CD38 sequence). Human CD38 has previously been studied and techniques for obtaining the extracellular domains of human CD38 in soluble form have also previously been described (see, for example, Daeglio *et al.*, J. Immunol.  
10 (1996) 156, 727-734).

The analogue of CD38 can inhibit the binding of CD38 to a FDC or DC. Therefore the amount of CD38 which can bind an FDC or DC in the presence of the analogue is decreased. This is because the analogue is able to bind CD38 ligand on the FDC or DC in a specific manner, and therefore competes with the CD38 for  
15 binding to CD38 ligand. The inhibition of binding can be determined using known binding assays, such as those discussed below. FDCs or DCs for use in such assays can be obtained by known methods, such as by sorting cells based on their ability to bind CD38.

Other binding characteristics of the analogue are generally also the same as  
20 CD38, and thus typically the analogue binds to antibodies specific for CD38 (e.g. specific for an extracellular portion or the ligand binding site of CD38), and thus inhibits binding of the CD38 to such an antibody.

The analogue retains the ability of CD38 to stimulate FDCs and/or DCs. Thus typically the analogue is able to cause an increase in the FDC networks and/or  
25 maturation of DCs (the maturation generally determined by measuring the upregulation of cell surface markers, such as MHC class I or II, or B7.2, on the DCs).

In one embodiment the analogue has ADP-ribosyl transferase activity. It typically has at least 10%, for example at least 20, 40, 60, 100, 150, 200% or more of the ADP-ribosyl transferase activity of CD38.

30           In one embodiment the analogue is a peptide or comprises 1, 2, 3 or more peptides typically joined together by peptide or non-peptide linkers. The analogue

may comprise sequence from two or more of the proteins discussed below either (e.g. fragments from the proteins) as a fusion protein or joined together by the linker(s).

A peptide analogue or a peptide which is present in an analogue typically has or comprises the same sequence as all or part of CD38, or a sequence which is homologous to part of or all of CD38.

The peptide may comprise a fragment of CD38 or a fragment of the homologous sequence, typically with a length of at least 10, 20, 30, 50 or 100 amino acids long. Such a fragment may be the extracellular portion or ligand binding site of CD38, or the equivalent sequence in the homologous sequence. In a preferred embodiment the fragment is the portion of CD38 which is encoded by the polynucleotide sequence which is amplified when SEQ ID NO:1 and 2 are used as primers in a PCR reaction in which a murine CD38 gene is used as template. The portion of human CD38 which is equivalent to such an amplified sequence (the 'equivalent' sequence is determined based on homology, e.g. using the PILEUP program in the UWGCG package) is also preferred and may be obtained in similar manner.

The peptide analogue or peptide which is present in the analogue may comprise sequence from other proteins (typically naturally occurring proteins). The sequence may be from an antibody (e.g. IgG), such as a Fab, (Fab)<sub>2</sub> fragment or Fc fragment. Such an antibody may be one which can bind CD38 ligand or a cell surface protein on a FDC or DC. In a preferred embodiment the peptide is a fusion protein which comprises an extracellular domain portion of CD38 and a non-CD38 sequence.

A preferred analogue comprises a fusion protein of the extracellular domain of CD38 (such as the murine fragment discussed above encoded by the sequence amplified by SEQ ID NO:1 and SEQ ID NO:2, or the equivalent human fragment) and the CH<sub>2</sub>CH<sub>3</sub> (Fc) domains of a human IgG1.

The sequence may be one that causes the analogue to associate with FDCs or DCs, such as the cell membranes of these cells or with proteins on the surface of these cells. Such sequence may be from a protein that binds CD38 ligand or that binds a cell surface protein of FDCs or DCs.

Typically the peptide analogue or peptide present in the analogue comprises 1, 2, 3, 4 or more modifications, which may be natural post-translational modifications or artificial modifications. The modifications are typically the same as the modifications (e.g. the glycosylation) present on natural CD38, typically at the same or equivalent positions. The modification may provide a chemical moiety (typically by substitution of a hydrogen, e.g. the hydrogen of a C-H bond in the peptide), such as an amino, acetyl, hydroxy or halogen (e.g. fluorine) group or carbohydrate group. Typically the modification is present on the N or C terminus of the peptide.

The analogue (or the peptide of the analogue) may comprise one or more non-natural amino acids, e.g. amino acids with a side chain different from natural amino acids. Generally, the non-natural amino acid will have an N terminus and/or a C terminus. The non-natural amino acid may be an L-amino acid.

The analogue typically has or comprises a part which has a shape, size, flexibility or electronic configuration which is substantially similar to CD38 or any of the fragments of CD38 discussed above. It is typically a derivative of CD38 or of such a fragment.

The analogue may be soluble or insoluble in water. The analogue may be capable of forming an oil-in-water or water-in-oil emulsion. The analogue may be capable of associating with a lipid membrane, such as a cell membrane.

The analogue is typically designed by computational means and then synthesised using methods known in the art. Alternatively the analogue can be selected from a library of compounds. The library may be a combinatorial library or a display library, such as a phage display library. Analogues are generally selected from the library based on their ability to mimic the binding characteristics and/or the ability to stimulate a FDC or DC. Thus they may be selected based on ability to bind a CD38 ligand (for example on a FDC or DC) or antibody which binds CD38.

#### Substances that provide CD38 or the analogue *in vivo*

As discussed above CD38 or an analogue can be used as an adjuvant *in vivo*. In one embodiment a substance capable of providing CD38 or the analogue *in vivo*

can be administered. Such a substance is included in the term 'analogue' herein.

The substance is typically a precursor of CD38 or the analogue and is capable of being modified (e.g. hydrolysed) *in vivo*, typically in a cell, to provide CD38 or the analogue.

5       The substance may be polynucleotide capable of being expressed to provide CD38, the analogue or the precursor. The polynucleotide is typically DNA or RNA, and is single or double stranded. The polynucleotide generally comprises sequence that encodes CD38, the analogue or the precursor. The coding sequence is typically operably linked to a control sequence (e.g. a promoter) capable of providing for  
10       expression of the polynucleotide. Thus typically the polynucleotide comprises 5' and 3' to the coding sequence sequences which aid expression, such as transcription and/or translation of the coding sequence.

15       The polynucleotide is typically capable of being expressed in the cells of any of the animals mentioned herein. The polynucleotide may be present in a viral or cellular vector.

20       The invention provides CD38 or an analogue for use as an adjuvant, in particular for stimulating a larger and/or longer lasting immune response against an antigen. The immune response may be an antibody response, or a CD4 or CD8 T cell response. CD38 or the analogue are also provided for increasing the length of time of B cell memory; and/or for increasing the size and/or number of FDC networks. In the case of an antibody response CD38 or the analogue are provided to increase the amount of specific antibodies and/or to increase the time for which such antibodies are produced. In the case of CD4 and CD8 cells the CD38 or analogue are  
25       provided to increase the numbers of such cells produced which are specific for the antigen.

      The invention provides CD38 or an analogue for use in causing maturation of DCs *in vivo*.

30       Generally in the method of causing maturation of a DC *in vitro*  $10^3$  to  $10^{12}$  (e.g.  $10^6$  to  $10^9$ ) DCs are present. Typically CD38 or the analogue are contacted with DCs at a concentration of  $10^{-2}$  to  $10^3$   $\mu\text{g/ml}$  (e.g. 1 to 10  $\mu\text{g/ml}$ ). The DCs are typically contacted with CD38 or the analogue for from 1 hour to 7 days (e.g. from 6

hours to 3 days). Generally the DCs will be contacted in conditions which support them (i.e. which keep them alive) and typically allow the DCs to grow or replicate.

In the method the DCs may also be contacted with other agents which bind proteins on the surface of the DCs (e.g. MHC class I or II molecules, or CD40)

5 and/or which stimulate the DCs. Suitable agents include antibodies to the surface proteins or the natural receptors of the surface proteins. Soluble (for example truncated) forms of the receptors may be used or cells may be used which naturally express the receptors on their surface.

The invention provides a vaccine which comprises CD38 or the analogue and  
10 an antigenic component. Such a vaccine is generally capable of stimulating an immune response to the antigenic component, such as any of the immune responses discussed herein. Thus the antigenic component will comprise antibody or T cell (e.g. CD4 or CD8) epitopes.

The vaccine may also comprise other adjuvants or delivery systems, such as  
15 adjuvants which stimulate a CD8 T cell response. In a preferred embodiment the antigenic component of the vaccine comprises a CD8 epitope.

The antigenic component is typically from a cancer cell (e.g. specific to a cancer cell, such as a neo-antigen) or a pathogen. The cancer or pathogen are typically ones which can be damaged or killed by an antibody or CD8 T cell  
20 response. The pathogen may be an intracellular or extracellular pathogen (e.g. a bacterium or virus).

The invention also provides an *ex vivo* mature DC which has been made using the method of the invention. As discussed below such a cell may be in an isolated or purified form. The cell may be in a composition which also comprises T cells or B  
25 cells. The cell may be in a composition of mononuclear cells (e.g. from peripheral blood). The mature DC may have been provided with antigen (such as any of the antigens, or proteins comprising any of the antigens, discussed above which are present in the antigenic component) when it was immature. Such a mature DC will generally comprise the antigen inside the cell (e.g. in the class I or II antigen  
30 processing pathway) or on its surface bound to MHC molecules.

Thus in one embodiment an immature DC is contacted with any of the

antigens or proteins discussed above (typically under conditions in which the DC is able to take in the antigen and protein and process it) and is then contacted with CD38 or the analogue to provide a mature DC of the invention. Such contacting may preferably be carried out simultaneously with provision of conditions such that the DCs correspond to DCs subject to T cell signalling. Such conditions may be conditions which mimic T cell signalling, e.g. contacting additionally with anti-CD40 and anti-MHC class II as described in the examples. In another embodiment, the antigen is provided inside the DC (in the same manner as discussed above with regards to substances that provide CD38 or the analogue *in vivo*). Thus the DC may be contacted with a precursor of the antigen or a polynucleotide that is capable of being expressed to provide the antigen.

Mature DCs produced in accordance with the invention typically have higher levels of MHC class I or B7.2 expression than an immature (e.g. splenic) DC, typically at least 2, 4, 6, 10 or more fold higher.

The invention provides a DC of the invention for use in a method of treating the human or animal body by therapy. In particular the DC is provided for use in stimulating a T cell response *in vivo*. Typically the response is a CD8 T cell response. In the case of the DC discussed above which was provided with antigen when immature the immune response is typically directed to the antigen used.

The invention also provides a vaccine comprising a DC of the invention.

The invention provides a method of stimulating T cells specific to an epitope *in vitro* comprising contacting the T cells with a DC of the invention under conditions in which the DC presents the epitope to the T cell. The method may be used to increase the numbers of such T cells. This may be for the purpose of administering them to a patient or to increase their numbers so that they can be detected. The T cells are CD4 or CD8 T cells.

The invention provides the ligand of CD38 as presented by FDC or DC in substantially isolated form (hereinafter referred to as CD38 ligand) and homologues of the ligand; and fragments thereof. Such homologues and fragments are included in the term 'ligand' herein. CD38 ligand may be obtained from a cell membrane (e.g. a FDC or DC cell membrane).

The ligands which are homologues or fragments are capable of binding CD38, and thus can typically inhibit the binding of CD38 to CD38 ligand. Preferred ligands retain the ability to cause CD38 mediated activation of FDCs or DCs when present in the cell membrane of FDCs or DCs.

5       The ligand is typically substantially free of FDC or DC cell membrane. The ligand may be substantially free of cell membrane or of cellular components. Such cell membranes may be those of prokaryotes or eukaryotes, mammals (such as humans, primates or rodents) or of the animal in which the particular CD38 naturally occurs.

10       The ligand may be identified (for example in its naturally occurring form) on a gel, such as under non-reducing conditions (see Example 2).

Ligands which are fragments preferably include the extracellular part of the natural CD38 ligand or the CD38 binding site (or the equivalent sequence in a homologue).

15       The ligand may or may not be able to bind CD38 or an analogue of the invention. The ligand may be soluble in water. The ligand may be able to associate with lipids, such as cell membranes.

20       The ligand is typically at least 5, 10, 20, 50, 100 or more amino acids in length. The ligand may be present in the form of a fusion protein which has additional amino acid sequence N and/or C terminal to the ligand sequence.

Polynucleotides of the invention also include sequences that encode a ligand of the invention. Such polynucleotides may also be DNA or RNA, and may be single or double stranded.

25       Polynucleotides of the invention can be incorporated into a recombinant replicable vector. The vector may be used to replicate the nucleic acid in a compatible host cell. Thus a polynucleotide of the invention may be made in a process comprising introducing a polynucleotide of the invention into a replicable vector, introducing the vector into a compatible host cell, and growing the host cell under conditions which bring about replication of the vector. The vector may be  
30       recovered from the host cell. Suitable host cells are described below in connection with expression vectors.

In one embodiment the polynucleotide of the invention in a vector is operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell.

5 The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

10 Such vectors may be transformed into a suitable host cell as described above to provide for expression of a ligand of the invention. Thus, in a further aspect the invention provides a process for preparing a ligand of the invention, which process comprises cultivating a host cell transformed or transfected with an expression vector as described above under conditions to provide for expression of the ligand, and recovering the expressed ligand.

15 The vectors may be for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene in the case of a bacterial plasmid or a neomycin resistance gene for a mammalian vector.

20 Vectors may be used *in vitro*, for example for the production of RNA or used to transfect or transform a host cell. The vector may also be adapted to be used *in vitro*, for example in a method of gene therapy.

25 A further embodiment of the invention provides host cells transformed or transfected with the vectors for the replication and expression of polynucleotides of the invention. The cells will be chosen to be compatible with the said vector and may for example be bacterial, yeast, insect or mammalian.

30 Promoters and other expression regulation signals may be selected to be compatible with the host cell for which the expression vector is designed. For example, yeast promoters include *S. cerevisiae* GAL4 and ADH promoters, *S. pombe* nmt1 and adh promoters. Mammalian promoters include the metallothionein promoter which can be induced in response to heavy metals such as cadmium. Most



preferably, the expression vectors are possible for use in insect or mammalian cells. For use in insect cells, strong baculovirus promoters such as the polyhedron promoter are preferred. For expression in mammalian cells, strong viral promoters such as the SV40 large T antigen promoter, a CMV promoter or an adenovirus promoter may  
5 also be used. All these promoters are readily available in the art.

Suitable cells include cells in which the abovementioned vectors may be expressed. These include microbial cells such as bacteria (e.g. *E. coli*), mammalian cells such as CHO cells, COS7 cells, P388 cells, HepG2 cells, KB cells, EL4 cells or HeLa cells, insect cells or yeast such as *Saccharomyces*. Baculovirus or vaccinia  
10 expression systems may be used.

The invention provides an antibody that binds CD38 ligand and inhibits the binding of CD38 to CD38 ligand. The antibody may be monoclonal or polyclonal. Such antibody may be produced in a process that comprises contacting CD38 with a population of B cells (*in vivo* or *ex vivo*), and then isolating antibody of the invention  
15 which is produced by such cells. As discussed below such antibody may be collected from the sera of animals to which CD38 ligand has been administered. Alternatively B cells (e.g. in the form of spleen) may be removed from such an animal, immortalised, and selected based on their ability to produce antibody that binds CD38 ligand. Antibody may be obtained from such selected cells.

An adjuvant may be identified in a process comprising determining whether a candidate substance binds specifically to CD38 ligand, specific binding indicating that the substance is an adjuvant. An adjuvant may be identified in a process comprising determining whether a candidate substance is capable of causing maturation of DCs, a substance which is capable of maturing DCs being an adjuvant.  
20 One aspect of the invention provides a method of identifying an adjuvant comprising contacting CD38 ligand with a candidate substance in the presence of CD38 or an analogue and determining whether said substance competes with CD38 or the analogue for binding to CD38 ligand and whether said substance is capable of maturing DCs. Adjuvants identified in these methods can be used in the *in vitro* and  
25 *in vivo* methods or vaccine discussed herein in the same manner as CD38 or the analogue.  
30

The invention also provides an inhibitor of CD38 ligand which specifically inhibits activation of CD38 ligand by CD38. Generally such an inhibitor binds CD38 ligand. It may have any of the structural characteristics discussed above in relation to the analogue of CD38. Thus the inhibitor may comprise a peptide with  
5 homology to CD38.

The inhibitor can be identified, for example, in a process comprising providing (contacting) a candidate substance to CD38 ligand in conditions in which in the absence of the candidate substance CD38 ligand would be activated, and determining whether the candidate substance causes inhibition of the activation of  
10 CD38 ligand.

The invention provides the inhibitor for use in a method of treatment of the human or animal body by therapy. In particular the inhibitor is provided for use in a method of immunotherapy. Such immunotherapy is generally immunosuppression, such as by inhibition of CD38 mediated activity of FDC or DC. Thus the ligand may  
15 be used to inhibit the maturation of DC or the activation of T cells by DC *in vivo*. The inhibitor may be used to inhibit the activity of FDC networks *in vivo*, such as by causing a decrease in the number of FDC networks. Thus the inhibitor may be used to decrease FDC mediated B cell activity, which would generally lead to a decreased amount of antibody being produced by the B cells and/or a decrease in the length of  
20 time of B cell memory.

Thus the inhibitor is provided for use in a method of treating a disease which is caused by an immune response. The disease may be an autoimmune disease. In one embodiment the disease is one in which the immune response is caused by a foreign agent (such as a pathogen), but the immune response has a deleterious effect  
25 on the host.

The inhibitor may also be used to treat DCs or FDCs *in vitro* in a process in which the DC or FDC is contacted with the inhibitor, generally in conditions which support (i.e. keep alive) the DC or FDC.

### 30 The substances and cells of the invention

The discussion below includes substances provided by the invention,

substances identified in the methods discussed herein, cells provided by the invention and cells which have been produced in the methods discussed herein.

5 The CD38, analogues, CD38 ligands, antibodies, polynucleotides (e.g. that encode analogues or ligands) or inhibitors (of CD38 ligand) discussed herein may be in substantially purified form. They may be in substantially isolated form, in which case they will generally comprise at least 70%, e.g. at least 80, 90, 95, 97 or 99% of the peptide, polynucleotide or dry mass in the preparation. These substances may be substantially free of cells or of cellular components (such as cell membranes).

10 The DCs of the invention or the T cells produced in the method of stimulating T cells may be in substantially purified form. They may be in substantially isolated form, in which case they will generally comprise at least 70%, e.g. at least 80, 90, 95, 97 or 99% of the cells or dry mass in the preparation.

15 Any of the above substances or cells may be in the form of a pharmaceutical composition which comprises the substance or cell and a pharmaceutically acceptable carrier or diluent. Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline.

20 CD38, the analogue or CD38 ligand may be in a soluble form or be associated with lipid, for example a lipid membrane, such as a vesicle or cell membrane. Thus these substances may be present on the surface of a cell, such as a cell on which CD38 or CD 38 ligand is or is not naturally expressed. The cell may be a T cell, B cell, macrophage or NK cell which may be intact or lysed. The CD38, analogue or CD38 ligand may be present in the a preparation made from such a cell, such as an extract (e.g. a partially purified extract) from such a cell.

25 Any of the above substances may be in any of the above forms when present in the vaccines of the invention or when used in the *in vitro* or *in vivo* methods of the invention.

#### Methods of producing antibodies

30 The antibodies mentioned herein may be produced by raising antibody in a host animal. Such antibodies will be specific to CD38 or to the products mentioned above which bind antibodies. CD38 or the products are referred to as the

'immunogen' below. Methods of producing monoclonal and polyclonal antibodies are well-known. A method for producing a polyclonal antibody comprises immunising a suitable host animal, for example an experimental animal, with the immunogen and isolating immunoglobulins from the serum. The animal may  
5 therefore be inoculated with the immunogen, blood subsequently removed from the animal and the IgG fraction purified. A method for producing a monoclonal antibody comprises immortalising cells which produce the desired antibody. Hybridoma cells may be produced by fusing spleen cells from an inoculated experimental animal with tumour cells (Kohler and Milstein (1975) *Nature* 256, 495-  
10 497).

An immortalized cell producing the desired antibody may be selected by a conventional procedure. The hybridomas may be grown in culture or injected intraperitoneally for formation of ascites fluid or into the blood stream of an allogenic host or immunocompromised host. Human antibody may be prepared by *in*  
15 *vitro* immunisation of human lymphocytes, followed by transformation of the lymphocytes with Epstein-Barr virus.

For the production of both monoclonal and polyclonal antibodies, the experimental animal is suitably a goat, rabbit, rat or mouse. If desired, the immunogen may be administered as a conjugate in which the immunogen is coupled,  
20 for example via a side chain of one of the amino acid residues, to a suitable carrier. The carrier molecule is typically a physiologically acceptable carrier. The antibody obtained may be isolated and, if desired, purified.

#### Homologous sequences

25 Peptides which have a homologous sequence to a given (original) peptide are discussed herein (for example peptide analogues or peptide(s) present in an analogue which are homologous to CD38, or homologues of CD38 ligand). The discussion below describes how such homologues may be related to the original peptide.

The homologous sequence is typically at least 70% homologous to the  
30 original peptide, preferably at least 80 or 90% and more preferably at least 95%, 97% or 99% homologous thereto, for example over a region of at least 20, preferably at

least 30, for instance at least 40, 60 or 100 or more contiguous amino acids. Methods of measuring protein homology are well known in the art and it will be understood by those of skill in the art that in the present context, homology is calculated on the basis of amino acid identity (sometimes referred to as "hard homology").

5 Homology can be measured using known methods. For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al* (1984) *Nucleic Acids Research* 12, p387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (typically on their default settings), for  
10 example as described in Altschul S. F. (1993) *J Mol Evol* 36:290-300; Altschul, S, F *et al* (1990) *J Mol Biol* 215:403-10.

Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pair (HSPs) by  
15 identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both  
20 directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached.  
25 The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci. USA* 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

30 The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci.*

USA 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

The homologous sequence typically differs from the original sequence by substitution, insertion or deletion. Generally from 1, 2, 3, 4 or more substitutions, deletions or insertions, for example over a region of at least 10, preferably at least 20, for instance at least 30, 40, 60 or 100 or more contiguous amino acids in the analogue. Thus the homologous sequence may differ from the original sequence by at least 2, 5, 10, 20, 30 or more substitutions, deletions or insertions.

The substitutions are preferably 'conservative'. These are defined according to the following Table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
AROMATIC		H F W Y

A polynucleotide sequence encoding the homologous peptide typically hybridises with a polynucleotide encoding the original peptide. It typically hybridises at a level significantly above background. The signal level generated by the interaction is typically at least 10 fold, preferably at least 100 fold, as intense as 'background' hybridisation. The intensity of interaction may be measured, for example, by radiolabelling the probe, e.g. with  $^{32}\text{P}$ . Selective hybridisation is typically achieved using conditions of medium to high stringency (for example

0.03M sodium chloride and 0.003M sodium citrate at from about 50°C to about 60°C).

### Binding

5 The binding between any two substances mentioned herein is typically a specific binding. The binding may be reversible or non-reversible binding.

Determination of binding, for example in the method of identifying an adjuvant, can be done by using any known binding assay. Typically whether or not a first substance binds to a second substance is done by determining whether the second substance is able to inhibit the binding of a substance known to bind the first substance (for example a specific antibody).

10 Binding may be determined by measuring a characteristic of any of the substances that changes upon binding, such as spectroscopic changes.

Binding between may be determined in a 'band shift' system, in which the retardation of a substance on a gel can be used to detect when it is bound to another substance. The binding may be determined in a competitive binding method.

### Administration

20 The substances and cells of the invention (in particular CD38, analogues, DCs, vaccines, T cells produced in the method of stimulating T cells, adjuvants identified in the method of identifying an adjuvant and inhibitors CD38 ligand of the invention) are referred to as the 'substances' below. The substances may be administered to a human or animal in need of treatment. The condition of the human or animal can thus be improved.

25 The invention provides the substances for use in a method of treating the human or animal body by therapy. Thus the substances are provided for use in immunotherapy. The substances are provided for use in the manufacture of an adjuvant (or an immunosuppressant in the case of an inhibitor of CD38 ligand) for use in immunotherapy. Thus the invention provides a method of treating a disease comprising administering a substance of the invention.

30 The substances may be combined with a pharmaceutically acceptable carrier or diluent to produce a pharmaceutical composition. Suitable carriers and diluents

include isotonic saline solutions, for example phosphate-buffered saline. The composition may be formulated for parenteral, intramuscular, intravenous, subcutaneous, or transdermal administration.

5 The dose at which the substance of the invention is administered to a patient will depend upon a variety of factors such as the age, weight and general condition of the patient, the condition that is being treated and the particular substance that is being administered. A suitable dose may however be from 0.1 to 100 mg/kg body weight such as 1 to 40 mg/kg body weight.

10 Substances which are polynucleotides can be administered directly as a naked nucleic acid construct. Uptake of naked nucleic acid constructs by mammalian cells is enhanced by several known transfection techniques for example those including the use of transfection agents. Example of these agents include cationic agents (for example calcium phosphate and DEAE-dextran) and lipofectants (for example lipofectam<sup>TM</sup> and transfectam<sup>TM</sup>). Typically, nucleic acid constructs are mixed  
15 with the transfection agent to produce a composition.

When the polynucleotide is delivered to cells by a viral vector, the amount of virus administered is in the range of from  $10^6$  to  $10^{10}$  pfu, preferably from  $10^7$  to  $10^9$  pfu, more preferably about  $10^8$  pfu for adenoviral vectors. When injected, typically 1-2 ml of virus in a pharmaceutically acceptable suitable carrier or diluent is  
20 administered. When the polynucleotide of the invention is administered as a naked nucleic acid, the amount of nucleic acid administered is typically in the range of from 1  $\mu$ g to 10 mg. The polynucleotide may be administered in a cellular vector.

In the case where the substance is a cell (or the substance is a polynucleotide in a cellular vector) then typically  $10^3$  to  $10^{12}$  cells are administered, such as  $10^6$  to  
25  $10^9$  cells.

The following Examples illustrate the invention:

#### Example 1

##### Materials and Methods

##### 30 Construction of soluble mouse CD38 Human IgG1 Fc Chimeric protein

The primers used to amplify the extracellular domain of mouse CD38 were 5'



(AGG CCG CGC TCA CTC CTG GTG GTG GTG TGG (SEQ ID NO:1)) and 3' (TAC TCA CGT ATT AAG TCT ACA CGA TGG GTG CTC (SEQ ID NO:2)). A cDNA library of splenic cells was used as template for PCR amplification. Each primer contained restriction sites that allowed subcloning into a vector that contained the sequence encoding the CH2CH3 (Fc) domains of human IgG1. The resulting plasmid was transfected into J558L cells. Soluble mouse construct (CD38 $\gamma$ 1) was purified from culture supernants using Protein G columns.

#### In vivo administration of soluble CD38 $\gamma$ 1

Mice were immunized with 50 $\mu$ g of soluble DNP-KLH (Calbiochem-Novobiochem, USA). Groups of 4 mice were injected i.v. with either CD38 $\gamma$ 1 or human IgG (Binding Site, Birmingham, UK) at 100 $\mu$ g/mouse/day for 4 days starting at the day of immunization.

#### Effect on FDC networks using tissue sections

Spleen and lymph nodes were collected from mice after 14 days and frozen for cryostat sections. Sections were fixed with either 2% paraformaldehyde or cold acetone. The sections were then stained with either FDC-M1 (FDC and tingible body macrophages), PNA (germinal centres), B220, CD3 or M115.4 (Anti-class II). And anti-Rat-Ig labelled with peroxidase. The number of FDC networks /x20 field, stained by FDC-M1, was counted in spleen sections of 4 treated and 4 untreated mice. Between 11 and 15 fields were counted per section/mouse and a t test used to determine statistical significance of differences in mean number of networks per field.

#### Distribution of the ligand for CD38

Mice were given SRBC and at the time of immunisation and after 1, 2, 5, 7 and 9 days the spleen and lymph nodes were collected. Tissue sections of spleen and lymph node from naïve and mice immunised with CRBC were labelled with the construct and anti-human IgG-Fc-peroxidase.

Sections were also double labelled with alkaline phosphatase labelled class II

antibody or F480. Some sections were treated with an rat anti-mouse Fc antibody known to block Fc binding and then labelled with the construct, but these sections could not be double labelled.

5     Flow cytometry

Purified DC (Wykes *et al* (1998), J.Immunol., 161, 1313-1319) were labelled with the construct and biotin labelled anti-human Ig and Streptavidin-FITC.

Isolation of immature splenic DC

10         Spleens from 8-10 week old C57BL/6 mice were digested in collagenase D and DNAase (Boehringer Mannheim, UK), RBC were lysed and cells incubated with KT3 (anti-CD3), anti-CD4 (TYS.191.1), anti- $\mu$  (B cells), 3D6 (marginal zone metalophillic macrophages) and biotinylated anti-Ig1 and anti-IgG2a antibodies (Binding Site Ltd., UK) for 1h at 4°C. Labelled cells were depleted by rosetting with  
15     anti-rat and anti-mouse Ig-coated SRBCs and layering over histopaque (Sigma, USA). Rosetting has the added advantage of depleting macrophages via their Fc receptors. Contaminating cells were depleted using MACS system (Miltenyi Biotec, Germany). The final DC-enriched population was examined by flow cytometry and immunocytochemistry and contained >95% DC based on morphology and expression  
20     of MHC Class II, CD11c (very weak expression) and CD 40 (very weak expression) with less than 1% T cells, B cells or macrophages. Dendritic cells were also produced using the adherence method described by Steinman and Cohn (1974) J. Exp. Med., 139, p380, which was known to produce mature DC. These adherent  
25     splenic DC were further treated to remove contaminating T, B cells and macrophages.

Immunoprecipitation and western Blotting

30         The ligand for CD38 was immunoprecipitated from DC pulsed with <sup>35</sup>S-methionine radiolabel for 4 hours. The ligand was then immunoprecipitated and run on SDS-PAGE gels as described in Wykes *et al* (1998) Euro. J. Immunol. 28, 548).

### Maturation of DC

Immature DC were treated with either 30µg/ml Human IgG1 (Binding Site Ltd., UK) or CD38γl. Other treatments included anti-CD40 (FGK-45) and anti-Class II (M115.4), or CD38γl, anti-CD40 and anti-Class II, CD38γl and anti-CD40 or LPS. All antibodies were used at 10µg/ml and the LPS at 50g/ml. The cells were cultured for 18 hours and labelled with antibodies to detect Class I (28.8.6S), class II (Cadairlane, Canada) B7.1 and B7.2.

### Example 2

#### Results

##### Characterisation of the construct

As indicated above, a chimeric protein of murine CD38 and the Fc portion of human IgG1 (CD38γl) was created and purified on protein G columns. The construct occurs predominantly as a dimer but some monomeric protein is seen under non-reducing conditions (Figure 1a). Under reducing conditions, a single band with an apparent molecular weight of 55kD is observed. The protein was shown to be glycosylated using N-endoglucosidase, with a reduction of approximately 5kD (Figure 1b).

##### Localization of ligand for CD38 in mice using Immunocytochemistry

CD38γl was used to localise the ligand for CD38 in the spleen and lymph node. Human IgG1 was used to detect any binding due to the Fc portion of Ig. Mice were given SRBC i.v. via the tail vein and the spleen and LN collected at day 0, 2, 5, 7, and 9. The sections were treated with the construct or human IgG1 and immunocytochemistry carried out.

While the human IgG bound occasional cells in the red pulp, the CD38γl bound to different cell types. Five to ten percent of single cells in the red pulp of the spleen expressed the ligand and MHC class II. The ligand was also weakly expressed by dendritic cells in the T cell area of the spleen. Due to weak detection of the ligand in tissue sections, flowcytometry of purified DC (Figure 2) was used to

demonstrate the ligand (Figure 3 and 4). The ligand was also found on networks of cells in the secondary follicles by days 3-5 following immunisation. The networks were FDC that co-expressed FDC-M1, a FDC specific marker. Antibody to Fc receptor was used to treat tissue sections prior to binding of the construct to rule out Fc mediated binding. We did not detect any ligand on T cells or B cells in the appropriate areas (flowcytometry did not show any evidence of binding to T cells and B cells either). There was some very weak staining of the vascular endothelium. The absence of any binding of the construct to cells expressing CD38 rules out homotypic adhesion.

There have been studies that suggested that human CD38 mediates adhesion of lymphoid cells to the endothelium via homotypic adhesion and that CD31 was possibly the receptor for CD38. However, in those studies western blotting was used and no direct binding between CD38 and the CD31 was shown.

#### Characterisation of the ligand for CD38

Fresh DC were lysed and run on a 3-15% gradient gel under non-reducing conditions. A western blot of the gel was then stained with the construct and anti-human Fc-HRP antibody (Figure 4). Bands of about 130kD, 65 and a doublet at 33kD were seen. A weak band of about 50 kD was also seen, especially in fresh immature DC. An irrelevant cell type or control antibody were not included in these experiments. <sup>35</sup>S-methionine/cystine labelling also detected these bands.

#### CD38 has a role in Germinal centre development in vivo

To identify a function for CD38, groups of mice were given soluble DNP-KLH either with human IgG1 or the CD38yl every day for 4 days. The spleens were collected after 14 days and the sections stained with various markers. Staining with antibodies to class II, B220 or CD3 did not show any change to the architecture or distribution of cells in lymphoid tissue. However, treatment of mice with CD38yl had markedly increased the numbers of FDC networks in GC, stained by the antibody FDC-M1 by at least 2.8 fold (t test,  $p < 0.001$ ). The apparent size of the germinal centres were also several fold larger as seen by PNA and FDC-M1 staining

and could account for the apparent increase in number of GC.

As B cells express CD38, we suggest that when B cells interact with CD38L on FDC in follicles, this interaction stimulates an expansion of the FDC network.

CD38L is the first molecule known to modulate FDC function which has implications for germinal centre and antibody memory development. Germinal centres are essential for the development of antibody memory and expansion and enhancement of this environment should in theory increase the numbers of memory B cells that develop. Furthermore, it has also been shown that antibody memory is dependent on FDC retaining unprocessed antigen on their surface via antibodies.

Thus a larger FDC network would also improve recall (memory) responses.

#### CD38 $\gamma$ 1 improves antibody memory

As a follow on from the previous observation that CD38g1 improved the size and numbers of germinal centres, we immunised 2 groups of Balb/c mice with 75 $\mu$ g DNP-KLH and 5d later gave one group 75 $\mu$ g CD38 $\gamma$ 1 and the remaining mice 75 $\mu$ g human IgG1 as a negative control. After 10 weeks, lymphoid cells were purified from the spleen of these mice and transferred to SCID mice of a B6 background, i.v. along with 10 $\mu$ g DNP-KLH. These SCID mice were also given spleen cells from Balb/c KLH primed mice to provide optimal T cell help. The mice were bled after 8 and 14d (data not shown) and tested for IgH<sup>a</sup> haplotype anti-DNP responses. Balb/c immunoglobulin is of IgH<sup>a</sup> haplotype and the B6 background is IgH<sup>b</sup>. Although SCID mice produce very little or no immunoglobulin, detection reagents specific for the IgH<sup>a</sup> haplotype confirmed that the immunoglobulin detected was of donor origin. The response after 8 days indicated memory responses since primary IgG responses take longer to develop.

The titres of IgM anti-DNP were low 8d after the transfer of memory cells in all mice. However, 5/5 mice given control antibody produced IgG1 anti-DNP antibody (Figure 5a) but only 1/5 mice produced a low titre IgG2a anti-DNP antibody (Figure 5b). In contrast, following treatment with CD38 $\gamma$ 1 under identical conditions, 5/5 mice produced IgG2a and IgG1 anti-DNP antibody (Figure 5a and b). The titres of IgG2a anti-DNP were 3-fold lower than IgG1 anti-DNP but 10-fold

higher than the single mouse that produced IgG2a anti-DNP.

CD38 has a role in DC maturation

To identify a function for CD38 ligand on DCs, fresh *ex vivo* immature DCs  
5 were purified (without adherence) and treated with CD38 $\gamma$ l alone or in combination  
with other antibodies. Human IgG1 was used to control for non-specific effects of Fc  
binding, although this was unlikely since cells expressing Fc receptors were removed  
using rosetting. LPS was used to show maximal maturation of DCs. Mature splenic  
DCs prepared by the adherence technique were used to compare maturity of treated  
10 cells. LPS treatment of fresh DCs increased expression of class I, class II and B7.2  
to levels comparable with mature DCs. However, B7.1 expression was not  
significantly affected.

Treatment of the DC with human  $\gamma$ l showed minimal changes in expression of  
all markers whereas treatment with anti-Class II and anti-CD40 (to mimic T cell  
15 signalling) induced a 28% increase in the numbers of cells expressing higher levels  
of class I. This treatment did not increase expression of any other marker. However,  
treatment with CD38 $\gamma$ l increased Class I expression modestly and in combination  
with anti-CD40, or anti-CD40 and Class II, increased class I expression to levels  
comparable to LPS treatment (Figure 4a). While the percentage of cells expressing  
20 "higher" class I levels was lower than mature splenic DC, the mean fluorescence  
intensity was higher. MHC class II levels on all treated cells were not markedly  
different from the mature splenic DC (Figure 4b). Moreover, B7.1 levels were  
reduced by all treatments especially CD38 $\gamma$ l in combination with anti-CD40 and  
class II when compared to human  $\gamma$ l and mature DC (Figure 4c). In contrast, the  
25 construct combination improved B7.2 expression to levels equal to LPS treatment  
and better than mature splenic DC (Figure 4d).

The implications for these observations are that these treated DC may have a  
role in CTL development. Firstly, the phenotype of LPS treated DC was comparable  
to mature splenic DC. Furthermore, mature splenic DC have been shown to prime  
30 CTL *in vivo*, suggesting that LPS- treated DC may also be capable of priming CTL.  
Since LPS induces IL-12 production by DC driving a TH1 cytokine profile essential

for CTL responses this may indeed be the case. Secondly, class I and B7.2 expression are essential to prime CTL and these were found on LPS and mature DC but not on immature DC which do not prime CTL. Thus, signalling of class II, CD40 and CD38L will mature DC, as determined by their in change in phenotypic expression, to a form known to prime CTL. In fact the higher B7.2 levels on these cells may suggest a pre-disposition to priming CTL.

The clinical implications are two-fold. Firstly, DC are required to prime CTL but a problem exists in that only immature DC will endocytose antigen. However, these DC will not prime CTL. Conversely, mature DC cannot take up antigen but can prime CTL. Therefore, in a clinical application immature DC, which are easier to obtain, could be pulsed with antigen *ex vivo* and then treated to mature them to a form which would prime CTL.

#### CD38 $\gamma$ 1 treated DC improve cell-specific cytotoxicity

Since studies have shown that the expression of class I and B7.2 were essential for the development of cytotoxic T cells, and our CD38 $\gamma$ 1 based treatment improved expression of these molecules, we tested these cells in vivo. Fresh DC were pulsed with P815 tumour lysate and treated with either human IgG1 control antibody or CD38 $\gamma$ 1/anti-Class II/anti-CD40 antibodies for 20 hours, washed and given to groups of 3 naïve DBA/2 mice. After 1 week, this process was repeated. After another week, the spleens were collected and cultured with irradiated P815 cells. After the third week, cell counts of spleen cells from CD38 $\gamma$ 1 treated DC were 3-fold higher than control treated mice. The absolute numbers of CD4, CD8 and B cells were increased (data not shown). The numbers of NK cells were also apparently increased. CTL assays showed that CTL and non-CTL mediated killing was also improved compared to untreated DC (Figure 6).

Claims

1. Use of CD38, or a portion thereof or analogue thereof which can inhibit the binding of CD38 to a non-follicular dendritic cell (DC) or a follicular dendritic cell (FDC) and which retains the ability of CD38 to stimulate a DC or FDC, for use in the manufacture of an adjuvant for use in immunotherapy.
2. Use according to claim 1 wherein the portion is an extracellular domain portion or the analogue is a fusion protein which comprises an extracellular domain portion of CD38 and a non-CD38 sequence.
3. Method of causing maturation of DCs comprising contacting DCs *ex vivo* with CD38 or a portion or analogue as defined in claim 1 or claim 2.
4. A method as claimed in claim 3 wherein said DCs are simultaneously with said contacting under conditions such that the DCs correspond to DCs subject to T cell signalling.
5. A vaccine comprising an antigenic component and an adjuvant component, wherein said adjuvant component comprises CD38 or a portion or analogue as defined in claim 1 or claim 2.
6. A vaccine according to claim 5 in which the antigenic component comprises a CD8 T cell epitope.
7. An *ex vivo* mature DC that has been made using the method of claim 3 or claim 4.
8. A DC according to claim 7 for use in a method of treating the human or animal body by therapy.



9. A DC according to claim 7 for use in stimulating a T cell response *in vivo*.
10. A vaccine comprising a DC according to claim 7.
11. A method of stimulating T cells specific to an epitope *in vitro* comprising contacting the T cells with a DC according to claim 7 under conditions in which the DC presents the epitope to the T cell.
12.
  - (i) The native ligand present on a FDC or DC which binds CD38 and which is substantially free of FDC or DC cell membrane, excluding CD31;
  - (ii) a protein which is at least 70% homologous to (i) and binds CD38; or
  - (iii) a fragment of (i) or (ii) which retains the ability to bind CD38.
13. A polynucleotide that encodes (i), (ii) or (iii) as defined in claim 12.
14. An antibody that binds (i), (ii) or (iii) as defined in claim 12 and inhibits the binding of CD38 to (i), (ii) or (iii).
15. A method of identifying an adjuvant comprising contacting the CD38 ligand present on FDCs or DCs, or (ii) or (iii) as defined in claim 12 with a candidate substance in the presence of CD38 or an analogue as defined in claim 1 and determining whether said substance competes with CD38 or the analogue for binding to the CD38 ligand, (ii) or (iii) and whether said substance is capable of maturing DCs.
16. A use, method or vaccine according to any one of claims 1 to 6 in which the analogue is an adjuvant identified in the method of claim 15.

17. An inhibitor of CD38 ligand as defined in claim 12, which specifically inhibits activation of said ligand by CD38.
18. An inhibitor according to claim 16 for use in immunosuppression.